UPTAKE, TURNOVER AND EXCRETION OF I-131 BY RAINBOW TROUT (SALMO GAIRDNERI)[1]

J. B. HUNN[2] AND P. O. FROMM

Department of Physiology and Pharmacology, Michigan State University, East Lansing, Michigan

One of the first observations on iodine metabolism in fishes was that of Marine and Lenhart (1910a, 1910b) who showed that addition of potassium iodide to water flowing past brook trout (*Salvelinus fontinalis*) could reduce the goitrous condition of these fish. Little additional information, exclusive of a few measurements on the iodine content of the thyroids of fish, was forthcoming until the advent of radioactive iodine. Since that time numerous studies have been made on iodine metabolism in fishes, with the bulk of the work being concentrated on thyroid physiology and biochemistry (see reviews by Pickford and Atz, 1957; Berg, Gorbman and Kobayashi, 1959; Leloup and Fontaine, 1960).

Studies of extra-thyroidal iodine have generally been limited to describing tissue other than the thyroid which concentrates iodine. Using injected doses of I-131, it has been established that the ovary of cyclostomes and teleosts, notochord of cyclostomes as well as gills and stomach of selachians will concentrate iodide (I-131) above the blood level. Maqsood, Reineke and Fromm (1961), using *in vitro* incubation, studied a number of tissues from rainbow trout and found that only the lower jaw (thyroid) concentrated I-131 above the level in the incubation medium. Few data, however, are available on the actual iodine content of nonthyroidal tissues exclusive of the blood. Robertson and Chaney (1953) reviewed the literature and have contributed values on the iodine content of a number of tissues of rainbow trout.

The level of injected radioiodine in the vascular compartment and its rate of turnover have been little studied in fish and concern blood levels and excretion rates. Leloup (1952) found that in mullet (*Mugil auratus*) the blood level of I-131 was 3.21% of the injected dose (per gram of blood) 8 hours after injection and 2.38% after 72 hours. Mullet pre-treated with thiourea (1 gram per liter of aquarium water) for 8 days had a blood level of 1.68% of the injected dose 8 hours after injection. Hickman (1959) reported the half-life of I-131 in the blood of salt-water flounder (*Platichthys stellatus*) ranged from 75 to 527 hours while that of the fresh-water flounder ranged from 203 to 279 hours.

Most studies of iodine excretion in fish have been concerned with determination of whole body loss; the findings showed great variation among different fishes. Leloup (1952) showed that mullet lose 24.7% of an injected dose of carrier-free I-131 in 24 hours and 45.3% in 72 hours; conger eels (*Conger*

---

[1] Published with the approval of the director, Michigan Agricultural Experiment Station, as Journal series No. 3203. This project was supported in part by the National Institutes of Health.

[2] Pre-doctoral NIH Research Fellow.

conger) lost 7% in 24 hours. Chavin (1956) found that goldfish (*Carassius auratus*) lost 65% of an injected dose in 24 hours, and Leloup and Fontaine (1960) indicate that rainbow trout at 20° C. lose 33.5% in 24 hours. It is of interest to note that Leloup (1952) found that mullet treated with thiourea excreted radioiodine at a greater rate than untreated controls and the blood level of I-131 remained below that of the controls.

The only information found on the routes and relative rates of excretion of iodide in fish is that obtained by E. K. Marshall, Jr. and published by Beiter (1933). In this study two aglomerular toadfish, *Opsanus tau*, were injected with NaI and after 23 hours 74% and 92% of the injected dose had been excreted by the gills while 0.67% and 1.1% was excreted by the kidney. Hickman (1959) has estimated that more than 80% of the I-131 injected into salt- and fresh-water starry flounder is excreted extrarenally.

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*) weighing from 75 to 173 grams, provided by the Michigan Department of Conservation, Wolf Lake Hatchery, were kept under constant illumination in static tanks of aged, aerated tap water at 12 ± 2° C. They were fed trout pellets every other day and the water was changed every third day. During experimental periods the fish were not fed.

Ten trout used in the 24-hour uptake study were injected intraperitoneally with carrier-free Na I-131 made up in distilled water. The injection volume was 0.1 ml. and contained 2.26 μc. of I-131. Five fish were also given injections of 20 mg. of NaSCN. The fish were then placed in separate aquaria containing 36 liters of aerated, aged, tap water. After 24 hours the fish were anesthetized with MS-222 and samples of blood, upper gill and lower jaw were obtained. Gill and jaw samples were rinsed in fish Ringer's solution, blotted dry and weighed to the nearest milligram, using a Roller-Smith balance. All samples were wet-ashed with nitric acid in five-dram plastic vials and diluted to a counting volume. Counts were made on 10-ml. aliquots of the aquarium water. Counting standards were made up in similar vials using 1/20 of the injected dose. All samples were counted using a two-inch thallium-treated NaI well scintillation detector, Model PHA-ICA pulse height analyzer and Model DS-1A decade scaler used as a slave scaler. Counting was done at the 5% level of error or less.

For blood turnover studies, 15 trout were injected with 3.87 μc. Na I-131 per fish, then evenly distributed among three aquaria, each containing 38 liters of aged tap water and equipped with a charcoal filter. Five fish were killed at 24, 72 and 120 hours and samples of blood and lower jaw taken. Blood samples were centrifuged at 2500 rpm for 20 minutes and plasma samples varying from 0.4 to 0.7 ml. removed. Plasma proteins were precipitated with 10% $ZnSO_4$ and 0.5 N NaOH and washed twice with 10 ml. of glass-distilled water. Precipitates, washes, tissue samples and aquaria water were counted as noted above.

Routes of excretion of I-131 were studied in two groups of trout. The first group contained radioiodide that had been accumulated by the fish during a 24-hour exposure to distilled water containing Na I-131. The second group was studied 24 hours after receiving an intraperitoneal injection of Na I-131. For this experiment the fish were anesthetized with MS-222 and a polyethylene

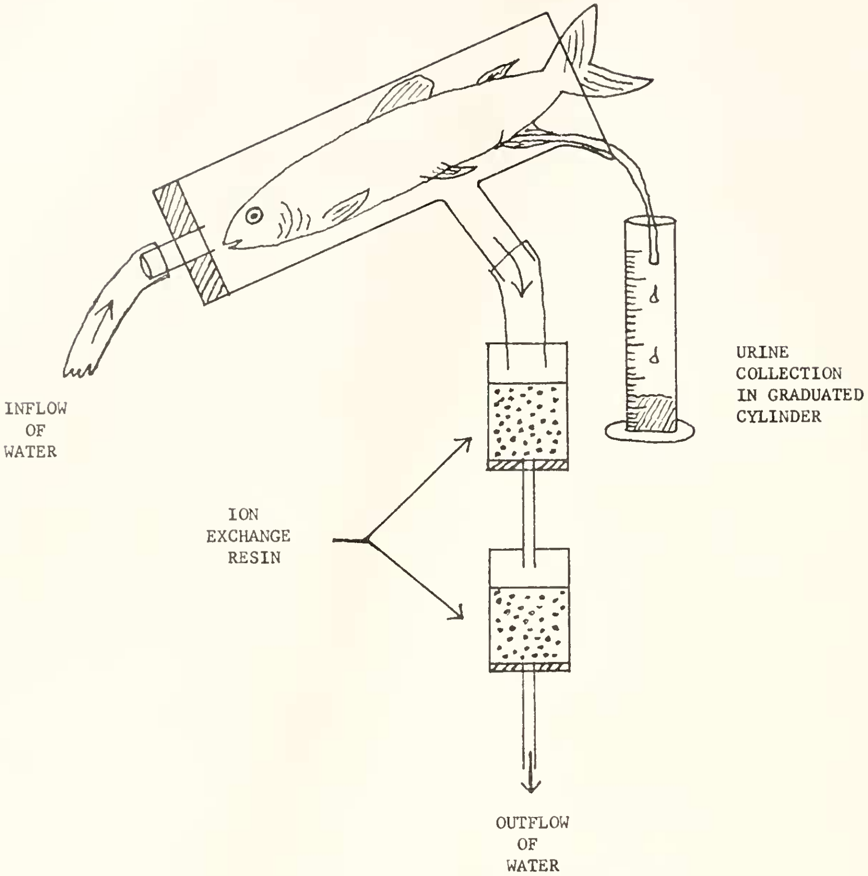

FIGURE 1. Distilled or tap water pumped over the gills of the experimental fish in the chamber, flowed out the side-arm into the two columns, each containing 10 grams of ion exchange resin, IRA 400. The cannula delivered urine into a 10-ml. graduate cylinder. To secure the animals in position damp cotton was tucked in around the tail of the fish.

cannula (Intramedic PE 60) with a flared tip was inserted into the urinary bladder. The fish was inserted into the urine-collecting apparatus (Fig. 1), damp cotton was placed around the posterior ends of the fish to keep them in place and the gills were bathed with tap or distilled water. At the end of the collection period the fish were again anesthetized with MS-222, blood samples taken, centrifuged and plasma drawn off. The radioactivity in the plasma, urine and resin samples was measured as noted above.

RESULTS

Data on the 24-hour tissue distribution of intraperitoneally injected Na I-131 (Table 1) indicate that the gills of trout do not accumulate iodide above the concentration found in the blood. In contrast, the lower jaw (thyroid) ex-

TABLE I

Twenty-four hour uptake of intraperitoneally injected I-131 by rainbow trout

Body weight $\bar{x}$ and range	n	% injected dose per gram tissue		
		Blood ($\bar{x}$ ± SE)	Gill ($\bar{x}$ ± SE)	Lower jaw ($\bar{x}$ ± SE)
Control				
129.7 (89.1–159.1)	5	3.10 ± 0.51	0.62 ± 0.06	11.20 ± 1.50
NaSCN (20 mg./fish)				
147.6 (98.1–172.6)	5	0.64 ± 0.16	0.26 ± 0.07	0.31 ± 0.07

hibits a definite facility for concentrating iodide which is inhibited by thiocyanate. In addition to blocking thyroidal uptake of radioiodine, administration of thiocyanate reduced the I-131 content of both blood and gill tissue. The I-131 content of the aquarium water 24 hours after injection showed that the rate of loss from the thiocyanate-treated fish was some three times greater than that of control fish (control 21.9% and NaSCN treated 69.7% of the injected dose).

Data on the relative distribution of I-131 in blood plasma following intraperitoneal injection are given in Table II. Less than 5% of the total radioiodide in the blood was found in the protein-bound fraction. The amount of protein-bound iodide decreased along with a decrease in the non-bound iodide until the fifth day after injection when it increased slightly, presumably the result of thyroidal production and or release of radioactive thyroxine. Thyroidal (lower jaw) radioiodine showed a continual rise during the five-day experimental period.

A very short biological half-life of 1.7 days (Fig. 2) indicates a very rapid rate of turnover for non-bound I-131 in trout plasma. The rate of disapppearance of I-131 from the plasma is a function of tissue exchange (addition and withdrawal) and excretion (renal and extrarenal).

The apparatus, as shown in Figure 1, was constructed to facilitate the study of routes and rates of excretion of I-131 from trout. The urine flow rates

TABLE II

Distribution and turnover of I-131 in the blood plasma of rainbow trout

	24 hours $\bar{x}$ ± SE*	72 hours $\bar{x}$ ± SE*	120 hours $\bar{x}$ ± SE*
% dose per gm. plasma	4.53 ± 0.61	2.35 ± 0.41	0.91 ± 0.25
% dose non-bound per gm. plasma	4.49 ± 0.60	2.33 ± 0.41	0.88 ± 0.11
% dose protein-bound per gm. plasma	0.037 ± 0.004	0.016 ± 0.003	0.026 ± 0.003
% I-131 protein-bound	0.83 ± 0.05	0.70 ± 0.11	2.90 ± 0.13
% dose per gm. lower jaw	7.87 ± 1.38	18.94 ± 4.82	31.79 ± 6.78
$\frac{\text{Lower jaw I-131}}{\text{Blood I-131}}$	1.82	7.89	36.36

* n = 5.

(Table III) were calculated from data obtained during collection periods ranging from 75 to 120 minutes. Urine collected ranged in volume from 1.2 to 1.9 ml. The flow values obtained are high compared to the values for rainbow trout given by Krogh (1939), Holmes (1961) and Fromm (1963). Despite this, the urine plasma ratios indicate that the trout kidney is quite efficient in reabsorbing iodide from the glomerular filtrate. The outflux of I-131 from the gills, measured simultaneously with urinary excretion, indicates that some 3.8 times more iodide is excreted by the kidney than by the gills per unit of time. Whether the urine gill excretion ratio remains constant or changes with iodide levels in the plasma is not known.

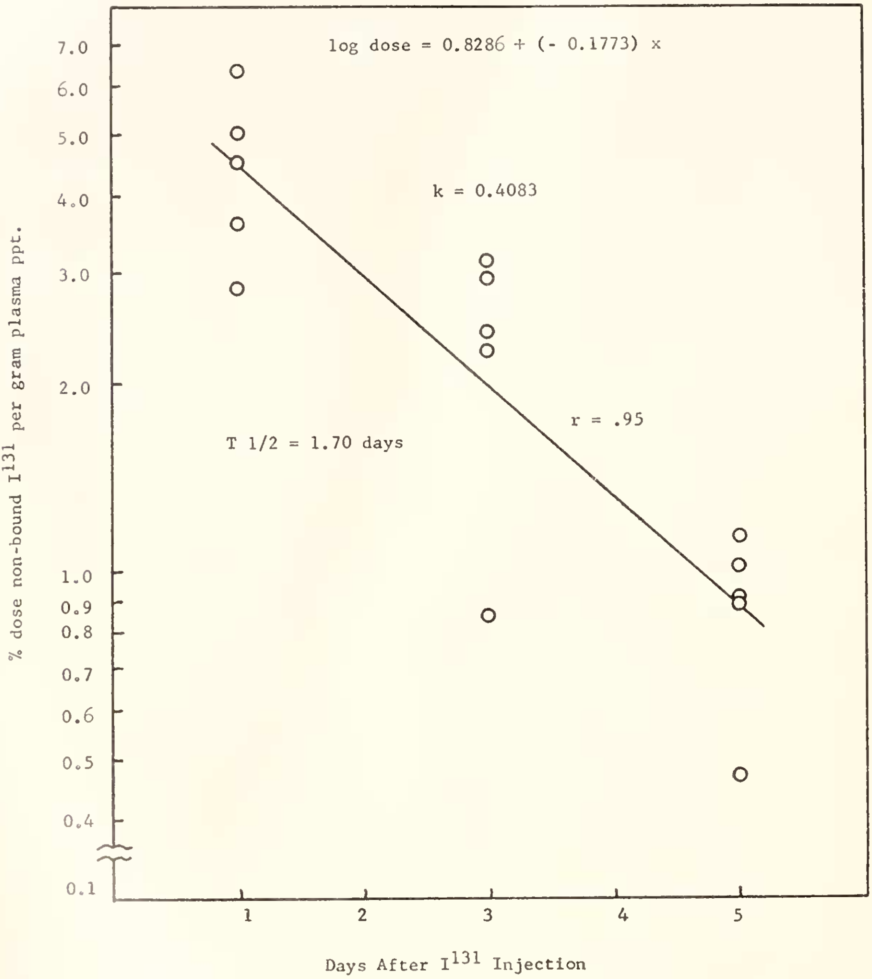

FIGURE 2. The biological half-life of non-bound I-131 in the blood plasma of rainbow trout.

TABLE III

Excretion of I-131 by rainbow trout

	Urine flow ml./Kg./day	I-131 urine/plasma ratio	I-131 clearance ml./hr.	I-131 urine/gill excretion ratio
Group I*				
1	129.6	0.30	0.22	3.92
2	232.2	0.07	0.06	2.74
3	160.8	0.27	0.17	6.29
4	211.2	0.34	0.25	3.05
5	190.0	0.10	0.10	3.61
Mean	181.0	0.22	0.16	3.92
Group II**				
1	176.4	0.08	0.04	4.0
2	151.2	0.09	0.06	4.5
3	153.6	0.10	0.06	—
4	192.0	0.07	0.04	2.66
Mean	168.3	0.08	0.05	3.72

* Group I exposed to I-131 in distilled water.
** Group II injected with I-131.

DISCUSSION

The effect of goitrogens on thyroidal uptake of I-131 in fishes is similar to that observed in mammals. The handling of the I-131 available due to blocking of the thyroid is, however, quite different. Bricker and Hlad (1955) have shown that administered SCN^- causes a marked increase in the plasma iodide I-131 levels in human hyperthyroid patients and a moderate rise in euthyroid patients. Similar increases in blood I-131 did not occur in trout treated with thiocyanate (Table I) and Leloup (1952) found no increase in blood I-131 of mullet and conger eels treated with thiourea and thiouracil, respectively. If the I-131 is not taken up by the thyroid and there is no increase in blood I-131, then the I-131 must be sequestered elsewhere or excreted.

I-131 levels in the aquarium water 24 hours after injection show that SCN^--treated trout lost three times as much I-131 as the controls. Leloup (1952) presented data showing that thiourea and thiouracil treatment of mullet and conger eels also increased excretion from two to eight times that of the controls. Bricker and Hlad (1955) have shown, however, that in humans SCN^- does not appreciably alter the I-131/inulin clearance ratios. If the fish kidney responds to SCN^- in a similar manner, then the increased excretion observed must have been extrarenal, possibly by way of the gills. The role of the gill in excreting halides (chloride) is well-established in marine fishes. Hickman (1959) and Leloup and Fontaine (1960) have shown that I-131 will accumulate in the gills at levels above that of the blood in both the starry flounder and dogfish (*Scyllium canicula*). This suggests that the gills may excrete iodide and this function is consistent with known chloride metabolism. Certainly the build-up of iodide in the gills could reverse the concentration gradient across the gills which would permit a passive loss of iodide from the fish to the environment. Both *in vitro* and *in vivo* studies have shown, however, that no build-up of iodide (I-131) occurs in the gills of fresh-water trout.

As shown in Figure 2, the biological half-life (T ½) of non-bound I-131 in the plasma of trout is 1.7 days. Hickman (1962), found that the T ½ (I-131) for whole blood was 202.6 and 279.4 hours for two fresh-water starry flounder, calculated from data obtained 30 and 70 hours after injection.

It should be noted (Table II) that almost all I-131 in the plasma is in the ionic form even five days post-injection. Although the amount of protein-bound I-131 is low, it is rather significant because it is non-hormonal. Leloup and Fontaine (1960) previously have shown that iodide will bind directly to trout plasma proteins *in vitro*. In the present study, by the fifth day, however, the I-131 in the protein-bound (PBI) fraction increases and this increase is probably due to the appearance of labeled thyroxine. Tong *et al.* (1961) indicate that labeled thyroxine was not detectable in the plasma of hagfish until the fourth day after injection. It is apparent from this and other work (Hickman, 1962) that great care should be taken in using plasma PBI as a parameter of thyroid activity in fish.

Our data, presented in Table III, indicate that per unit time some 3.8 times more I-131 is excreted in the urine than is lost by the gills in fresh-water rainbow trout. Observations by Marshall (see Beiter, 1933) indicated more iodide was excreted by the gill than by the kidney in toadfish. These conflicting results are not entirely unexpected since the trout has a glomerular kidney while that of the toadfish is aglomerular. Also the activity of the gills in osmoregulation is quite different in the two species.

Since the I-131 in the plasma of trout is mostly ionic, the glomerular filtrate should have about the same concentration of I-131 as the plasma. The urine plasma ratios of I-131 indicate that the I-131 of the glomerular filtrate is mostly reabsorbed. Bricker and Hlad (1955), Giebisch *et al.* (1956) and Williamson *et al.* (1962) have shown that in mammals, iodide reabsorption is a passive diffusion process taking place mainly in the proximal tubule. The rainbow trout kidney has a proximal tubule; however, the lack of correlative measurements precludes any conclusion as to the nature of the reabsorptive process in trout.

Another parameter of renal function, I-131 clearance, was determined. In studies of the clearance of any substance by the kidney, the plasma level of that substance should remain reasonably constant throughout the test period. The short test period, and the fact that little I-131 was lost from the fish indicate that this condition was fulfilled in our studies. Our experiments were designed to get information on the maximal (diuretic) and normal values for I-131 clearance by the kidney of a fresh-water salmonid. Diuresis was induced by exposing trout to distilled water, thereby increasing the osmotic gradient across the gills. Tap water was used as a control since its composition approximates that of Michigan trout waters. Published values for urine flow in rainbow trout range from 60–110 ml. kg./day. Holmes and McBean (1963) have found the GFR (glomerular filtration rate) of rainbow trout at 12° C. to be 170 ml./kg./day; thus, some 30%–60% of the glomerular filtrate is excreted per day. Values for urine flow obtained in this study suggest that the trout were diuretic and therefore the measured values can be considered to be near maximal.

The average iodide clearance for trout exposed to distilled water was three times that of trout injected with Na I-131 and then exposed to tap water (Table III). The increased clearance may have been due to differences in uptake (the distilled water animals had accumulated I-131 from distilled water which contained a small amount of Na I-131, whereas the tap water animals received an intraperitoneal injection of Na I-131) or more probably due to the greater diuretic stress imposed on the fish in distilled water.

It is quite apparent that the kidney of fresh-water trout is of major importance for the excretion of iodide. Reabsorption of filtered iodide is relatively efficient, as indicated by low urine/plasma ratio; however, the efficiency is somewhat less than that for chloride. Fromm (1963) has obtained a chloride clearance value of 6.8 ml./kg./day for rainbow trout whereas an iodide clearance of 12.6 ml./kg. day was obtained in the present study.

The authors wish to express their gratitude to Dr. E. P. Reineke and Dr. W. D. Collings for their helpful suggestions.

SUMMARY

1. NaSCN inhibits I-131 uptake by the lower jaw (thyroid) and increases the rate of loss of I-131 from trout, presumably by way of the gills.

2. The biological half-life (T ½) of ionic I-131 in the blood plasma of trout is 1.7 days.

3. I-131 is excreted in the urine and by the gills, with the urinary loss being 3.8 times that of the gill per unit time.

4. The I-131 urine/ plasma ratios indicate that the trout kidney absorbs much of the filtered iodide.

LITERATURE CITED

Berg, O., A. Gorbman and H. Kobayashi, 1959. The thyroid hormones in invertebrates and lower vertebrates. *In:* Comparative Endocrinology (A. Gorbman, Ed.): pp. 302–319.

Bricker, N. S., and C. J. Hlad, Jr., 1955. Observations on the mechanism of the renal clearance of I-131. *J. Clin. Invest.*, **34**: 1057–1072.

Chavin, W., 1956. Thyroid distribution and function in the goldfish, *Carassius auratus. J. Exp. Zool.*, **133**: 259–275.

Fromm, P. O., 1963. Studies on renal and extra-renal excretion in a fresh-water teleost. *Comp. Biochem. Physiol.*, **10**: 121–126.

Giebisch, G., M. B. McLeod and F. Kavaler, 1956. Renal excretion of radioiodide in the dog. *Amer. J. Physiol.*, **187**: 529–535.

Hickman, C. P., Jr., 1959. The osmoregulatory role of the thyroid gland in the starry flounder, *Platichthys stellatus. Canad. J. Zool.*, **37**: 997–1060.

Hickman, C. P., Jr., 1962. Influence of environment on the metabolism of iodide in fish. *Gen. Comp. Endocrinol., Suppl.*, **1**: 48–62.

Holmes, R. M., 1961. Kidney function in migrating salmonids. *Rep. Challenger Soc. Camb.*, **3**: 23.

Holmes, W. N. and R. L. McBean, 1963. Studies on the glomerular filtration rate of rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.*, **40**: 335–341.

Krogh, A., 1939. Osmotic Regulation in Aquatic Animals. Cambridge University Press.

Leloup, J., 1952. Action des antithyroïdiens sur la fixation de l'iode radioactif et la synthèse de la thyroxine dans la thyroide de deux téléostéens marins. *C. R. Soc. Biol.*, **146**: 1017–1020.

Leloup, J., and M. Fontaine, 1960. Iodine metabolism in lower vertebrates. *Ann. N. Y. Acad. Sci.*, **86**: 316–353.

Maqsood, M., E. P. Reineke and P. O. Fromm, 1961. *In vitro* I-131 uptake by tissues of rainbow trout. *Physiol. Zoöl.*, **34**: 34–38.

Marine, D., and C. H. Lenhart, 1910a. On the occurrence of goitre (active thyroid hyperplasia) in fish. *Bull. Johns Hopkins Hosp.*, **21**: 95–98.

Marine, D., and C. H. Lenhart, 1910b. Observations and experiments on the so-called thyroid carcinoma of brook trout (*Salvelinus fontinalis*) and its relation to ordinary goitre. I. *J. Exp. Med.*, **12**: 311–337.

Marshall, E. K.; in Beiter, R. N., 1933. Further studies concerning the action of diuretics upon the aglomerular kidney. *J. Pharmacol. Exper. Therap.*, **49**: 250–256.

Pickford, G. E., and J. W. Atz, 1957. The Physiology of the Pituitary Gland of Fishes. N. Y. Zoological Soc., New York, N. Y.

Robertson, O. H., and A. L. Chaney, 1953. Thyroid hyperplasia and tissue iodine content in spawning rainbow trout: A comparative study of Lake Michigan and California sea-run trout. *Physiol. Zoöl.*, **26**: 328–340.

Tong, W., P. Kerkof and I. L. Chaikoff, 1961. I-131 utilization by thyroid tissue of hagfish. *Biochem. Biophys. Acta*, **52**: 299–304.

Williamson, H. E., J. R. Scranton, F. N. Marshall and N. S. Halmi, 1962. Stop flow analysis of renal tubular site of reabsorption of radioiodide in dogs. *Proc. Soc. Exp. Biol. Med.*, **109**: 23–26.